distribution. This improvement in resolution directly relates to the amount of information that can be recorded in the data set. Also, the sidelobe intensity of the scanning confocal microscope is reduced from approximately 1% in conventional microscopy to 0.01% in confocal microscopy, again see, , Agard, D. A., Hiraoka, Y., Shaw, P., Sedat, J. W., Methods in Cell Biology—Volume 30, Chapter 13 "Fluorescence Microscopy in Three Dimensions," San Diego, Calif., Academic Press, pp. 353–377, 1989. This greatly reduces the convolution effects thus essentially eliminating scattering. As a result, the dynamic range of intensity is increased two orders of magnitude, from 100:1 in conventional microscopy to 10,000:1 in a confocal system.

In fluorescence microscopy the point resolution is broadened due to the increased wavelength of the fluorescent light compared to the shorter wavelength of the laser illumination light. Accordingly, resolution of the confocal microscope is somewhat reduced as a result of fluorescence, but this is also a problem with conventional fluorescence microscopy as well.

While, the illumination pinhole in a confocal microscope can be small enough so that the illumination is essentially a point source, the detection pinholes must typically be larger to collect enough light from weakly fluorescing objects. Consequently, the advantages gained in reducing convolution effects are somewhat diminished since the detected light is a result of the convolution with the pinhole aperture.

Image acquisition with the confocal microscope must be carried out on a point by point basis, either by scanning a stationary specimen or by moving the specimen with respect to the confocal pinholes. In any case, image acquisition is presently a relatively slow process.

Confocal microscopes suffer from photobleaching and phototoxicity. This occurs due to the high intensity laser light being focused on a point in the specimen. The fluorescent dyes contribute to produce toxic oxygen radicals within the cell. As is well known, photobleaching can be minimized with the use of antioxidants, however, this is chemically intrusive, thereby limiting the study of cell processes.

In light of the foregoing, when attempting to image dynamic cell processes, a three-dimensional wide-field microscope employing digital signal processing techniques is often preferred. Nevertheless, it is otherwise preferred to acquire high resolution images without the need for employing digital signal processing techniques using confocal microscopes.

Microscopy systems are used to acquire images with sub-micron resolution. In general, optical microscopy is limited in resolution by the wavelength of light (λ) and the numerical aperture of the lens (N.A.=$\eta \sin \alpha$), whereby the minimum resolvable distance between distinct points can be expressed as:

$$d_{\min} \approx \frac{\lambda}{\eta \sin\alpha} \tag{1}$$

where η is the index of refraction and α is a lens cone ½ angle, see Gray, P., Slayter, E. M., The Encyclopedia of Microscopy and Microtechnique, New York, Van Nostrand Reinhold, pp. 382–389, 1973, which is incorporated herein by reference. This limitation is due to lens aberrations causing out of focus blur and the effects of the convolution of an image with an aperture of a system. The convolution effects are limited to two-dimensions only. Therefore, the blurriness associated with a three-dimensional data set is due to convolution effects in two-dimensions (i.e., x-y image planes) and aberrations in three-dimensions. By way of example, for a circular aperture, the convolution of a point is the Airy disc pattern (i.e., a function of J_1 which is a Bessel function of the first kind, first order). As is readily apparent, a further analysis leads to the Rayleigh resolution criteria which defines the minimum resolvable distance between two points as the separation of the peak intensities where the peak intensity of the first point corresponds with the first minimum of the second point, assuming a circular aperture. See, for example: Cherry, R. J., New Techniques of Optical Microscopy and Microspectroscopy, Boca Raton, Fla., CRC Press (Macmillan Press), pp. 31–43, 1991: Goodman, J. W., Introduction to Fourier Optics, New York, McGraw-Hill, pp. 101–136, 1988; and Gray, P., Slayter, E. M., The Encyclopedia of Microscopy and Microtechnique, New York, Van Nostrand Reinhold, pp. 382–389, 1973. The Rayleigh resolution criteria can be expressed as:

$$\text{Rayleigh } d_{\min} = \frac{1.22\,\lambda}{2\,N.A.} \tag{2}$$

or, expressed in terms of the highest spatial frequency, f_p, as:

$$\text{Rayleigh } d_{min}=1.22/f_p \tag{3}$$

where f_p is defined as:

$$f_p=2\eta \sin \alpha/\lambda \tag{4}$$

It will be appreciated that the Rayleigh resolution criteria only addresses the convolution effects and is a two-dimensional phenomenon. However, aberrations affect the image quality in three-dimensions. The five types of aberrations in monochromatic systems (see, Smith, W. J., Modern Optical Engineering, New York, McGraw-Hill, pp. 49–58, 1966), commonly referred to as the Seidel aberrations are: spherical, astigmatism, curvature of field, distortion, and coma. If white light is used in the imaging process then the additional effects of chromatic aberration also degrade image quality. Lens are generally designed to greatly minimized aberrations. The convolution effects can be minimized as well by using a confocal microscope. Unfortunately, a confocal microscope would cause photobleaching and photodamage to cells thereby limiting its widespread application, as is well known. Consequently, three-dimensional wide-field microscopy of the prior art relies on digital signal processing (DSP) techniques to deconvolve an image data and remove out-of-focus blurring.

Prior attempts to use deconvolution have been plagued by poor signal-to-noise (S/N) results. One of the suspected reasons for these poor results are less than ideal convergence to the optimum deconvolution transfer function and the inability to adequately suppress noise. Typically, a Wiener filter implemented in the frequency-domain is used, but Wiener filters are notorious for not adequately suppressing noise, see Agard, D. A., Hiraoka, Y., Shaw, P., Sedat, J. W., Methods in Cell Biology—Volume 30, Chapter 13 "Fluorescence Microscopy in Three Dimensions," San Diego, Calif., Academic Press, pp. 353–377, 1989 and Cherry, R. J., New Techniques of Optical Microscopy and Microspectroscopy, Boca Raton, Fla., CRC Press (Macmillan Press), pp. 31–43, 1991. One reason for this is that the noise characteristics must be known a priori and are assumed to be statistically stationary. Any non-stationarities present in the data have an adverse effect on the signal-to-noise ratio (S/N). The non-stationarities can be dealt with by using more sophisticated algorithms, e.g., Kalman filters do